July 1, 2003

Joseph J. Merenda
Director, Office of Science Coordination and Policy
US Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Re: **Docket Control Number OPPTS-2003-0016.**
Issues Pertaining to the EPA's EDMVS One-generation and Multi-generation Rat Reproduction Study Design and Validation

Dear Dr. Merenda:

CropLife America (CLA) appreciates the opportunity to provide comments to EPA on the study design and validation for the one-generation and two-generation rat reproduction tests. CLA represents the manufacturers, formulators, and distributors of plant science solutions for agriculture and pest management in the United States. Herein, we are summarizing key points from CLA's presentation at the June 5, 2003 public comments session (see attached slides). We are also providing our recommendations on the June 5-6, 2003 EDMVS discussions.

CLA supports the development of a validated, scientifically sound endocrine prioritization and screening/ testing program with the opportunity for broad stakeholder involvement. EPA and the public have benefited from the open and transparent processes of both the Endocrine Disruption Screening and Testing Advisory Committee (EDSTAC) and the Endocrine Disruption Methods Validation Subcommittee (EDMVS). CLA has been very pleased to advise EPA in these forums, and we remain strongly committed to working with the Agency and other stakeholders to complete the process.

The development of appropriate study design to revise endpoints for 1998 OPPTS Guideline 870.3800 two-generation rat reproduction test is an important issue for all stakeholders. The two-generation rat reproduction test provides information critical for registration and re-registration of pesticide products, as required by the Federal Insecticide, Rodenticide and Fungicide Act (FIFRA), and to inform the No Observable Effect Level (NOEL) for pesticide risk assessment. The multi-generation rat test was also recommended by EDSTAC as a Tier 2 test to confirm potential endocrine disruption from Tier 1 screening. During 1998-2000, EDTSAC and the post-EDSTAC Standardization and Validation Task Force (SVTF) provided recommendations on specific and targeted estrogen, androgen and thyroid (EAT) endpoints to revise the 1998 OPPTS Guideline 870.3800.

CLA is concerned about the continual and unnecessary delays in finalizing a protocol and initiating validation of the rat multi-generation test. We believe that the EDSTAC and SVTF recommendations were based on consideration of critical data gaps in order to provide a more comprehensive evaluation of relevant and potentially adverse EAT effects. The current two-generation rat reproduction test is already complex and resource intensive. The test typically costs more than $500,000 and requires more than 3,000 rats and at least 18 months to complete the in-life and pathology measurements (quality assurance and report writing require additional time). The SVTF recommended upgrades to the 1998 protocol excluded redundant measurements of unnecessary details about already obvious EAT effects.

In FFDCA §408(p) as amended by the Food Quality Protection Act (FQPA), the Agency is mandated to develop an endocrine testing program, including the revised Tier 2 rat two-generation reproduction test (in its entirety, with any added endpoints), that is "appropriately validated." We are concerned from the discussions at the June 6, 2003 meeting of the EDMVS that the Agency may be deferring or short cutting the validation process for this test. We emphasize that the addition of any new endpoints to the rat multi-generation test must preserve the sensitivity of this test to confirm results from Tier 1 screening and/or eliminate concerns about adverse endocrine effects in the absence of Tier 1 screening data. More often than not, in assessing the potential adversity of an endocrine-related effect, there has been a lack of consensus on study design, interpretation of research results and reproducibility of data within and between laboratories. FQPA and Safe Drinking Water (SDWA) Amendments of 1996 stipulated that the tests for estrogenic and other endocrine effects must be fully validated to avoid this problem and to ensure reliability, consistency and data quality for risk assessment purposes.

Additionally, the efforts of the EDMVS appears to be side-tracked, to the detriment of the timely validation, by investigations of unnecessary anti-androgenic endpoints, whose overall effect would not have changed conclusions reached using the 1998 OPPTS Guideline 870.3800 two-generation rat reproduction test, and generational extensions that are more appropriate for higher-tier testing and/or mechanistic investigations. In our opinion, various stakeholders and the public are not being served by these unnecessary diversions that have had the net effect of second-guessing the EDSTAC/SVTF recommendations and slowing down the process of finalizing a two-generation rat reproduction protocol for validation.

CLA urges EPA to initiate validation of the 1998 OPPTS Guideline 870.3800 two-generation rat reproduction test with the addition of selected endpoints to address critical gaps in that are relevant for human health issues. The process as stated on Page 22 of the "Mammalian two-generation assay validation: History, Plan and Questions for the Endocrine Disruptor Methods Validation Subcommittee, June 6, 2003" (the "Report") would take approximately 22 months. This timeline could coincide with the completion of Tier 1 screen validation and ensure synchronization of mammalian screening and testing. Moreover, the availability of a validated Tier 2 two-generation rat reproduction test to address positives from Tier 1 screening could also alleviate the development and impact of pesticide priority screening lists and potential product de-selection issues.

The following summarizes CLA position for appropriate endpoints and generational extensions to the 1998 OPPTS Guideline 870.3800.

- CLA generally supports recommendations, with the exception of the superfluous and technically difficult challenge of weighing the ventral and dorsolateral rat prostrate gland lobes separately, as summarized Attachment 1 the "Report." See pgs. 13-14 and option 1 pages 22-23. In addition, we recommend that the assessment of thyroid parameters in the two-generation rat reproduction test be triggered endpoints. When results of other studies (e.g., 28-day or 90-day exposure studies) indicate that the thyroid is not a target organ, assessment of thyroid endpoints is unnecessary and redundant.

- The practicality and feasibility of adding additional endpoints to a large-scale and complex study, such as the two-generation rat reproduction test, must be carefully investigated from a technical perspective and whether these additional endpoints would add value to the risk assessment process (e.g., measuring the length of the gubernacular cords). CLA urges the Agency to differentiate endpoints that should be conducted routinely from those endpoints that should triggered. Based on current data, there is no strong indication that the current two-generation rat reproduction study protocol would miss significant anti-androgenic effects.

- Retained nipples in male rodents have no known effect on function or health and may not necessarily predict other overt anti-androgenic effects, e.g., hypospadia. Moreover, since human males retain their nipples, the value of this endpoint as an indication for human health concern is also very questionable.

- CLA strongly emphasizes that there is insufficient evidence to justify supporting extension of the F1 generation and the use of additional animals into a two-generation rat reproduction test. As stated already, we strongly contend that identification of adverse anti-androgenic effects is obvious from androgenic endpoints included in the 1998 OPPTS Guideline 870.3800. Moreover, to proceed with validation including the F1 extension adds unnecessary time to the validation process (estimated 22 months versus 44 to 56 months).

- CLA would support that, on case-by-case basis, a single-generation reproduction study which could have utility in addressing data gaps for older rat reproduction studies (non-1998 OPPTS Guideline 870.3800) and to avoid the unnecessary expenditures of resources (cost, animals, EPA review time, etc.) of repeating a two-generation rat reproduction tests for all pesticide active ingredients.

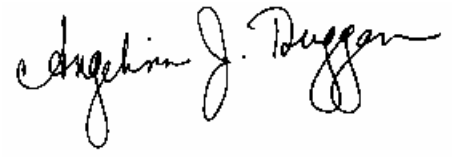
- CLA believes that the proposed *in utero* lactation study and any other F1 extended one-generation rat reproduction study should also be carefully assessed and held to the same standards of validation as other Tier 1 screens and Tier 2 endocrine tests. These are separate and complex tests; it should not be assumed that they are *de facto* validated simply because they are half a two-generation rat reproduction test. Before proceeding, value to risk assessment should be the strongest criteria in determining whether the expense and use of animals justifies validating these proposed tests.

- CLA also stresses the need for thoroughness in evaluating the outcomes and value of added endpoints and extensions to one-generation rat reproduction studies. CLA is very concerned that there was a decision not to retain tissues for histopathology in the RTI one-generation extension study that was presented to the EDMVS on June 6, 2003. While lack of histopathology may at times be acceptable for mechanistic studies, discarding tissues is inconsistent with the GLP standard that registrants must adhere to in submitting guideline registration studies to EPA. In our opinion, the histopathology data gaps in the RTI one-generation rat reproduction study are serious omissions that diminish the capability to understand whether the morphological differences/observations for PND 21 versus PND 95 in treated versus untreated animals were actually adverse.

In summary, CropLife America urges EPA to proceed with validating the two-generation rat reproduction test without further delay, as recommended by the SVTF (with the exceptions as noted above.) Progress on this effort should not be hampered or delayed by investigations into the value and utility of one-generation extensions for the two-generation rat reproduction study. Research on one-generation rat reproduction studies should continue as EPA has the funds to do so. However, before implementing either the *in utero* lactation and/or extended one-generation rat reproduction study for anti-androgens, EPA should not overlook critical steps in fully validating these assays and addressing how these tests would add value to risk assessment.

CropLife America would appreciate the opportunity to meet with you and the EPA OSCP and OPP staff to discuss our positions and recommendations.

Sincerely yours,



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Attachment: CLA Slides, 6/5/2003, 3 pages

Issues Pertaining to One-generation and two-generation Rat Reproduction Study design and Validation

Angelina J. Duggan, Ph.D.

CropLife America
EDMVS
June 5, 2003

CLA Supports 2-Gen Rat Reproduction as a Tier2 Test

- **As a definitive test following Tier1 screening**
- **As a Tier 1 by-pass option – by choice and for pesticides**
- **No short cuts on validation**

CLA Supports Validation 2-Gen Rat Reproduction as Tier2 Test

- **Critical that this test is sufficiently sensitive and robust to either confirm or determine/eliminate concerns for adverse endocrine effects**
  - Differentiate endocrine effects from dose related systemic toxicity.
- **CLA urges EPA/EDMVS to consider relevance/value of <u>all</u> additional endpoints**
- **Differentiate endpoints that should be conducted routinely vs. those that should be triggered**
  - E.G. thyroid hormones & histopathology
  - Extensive F1 adult male necropsy unless triggered by anti-androgenic observations in weaning.
- **Critical that the revised Tier 2 test, with all new endpoints, is demonstrated and validated in its entirety**

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Rat 2-Gen Reproduction Test Is Already Complex and Resource Intensive

- **Typically, the test uses 3,040 rats, 18 months (in-life and follow-on pathology only) and costs >> $500K**
  - Typically conducted after 90 Day previously to inform doses and target tissues
- **Support case-by case evaluation whether it is necessary to repeat or address gaps**
- **Other alternatives:**
  - 1-gen repro to bridge 2-gen data gaps
  - Thyroid endpoints in short-term/subchronic tests, pubertal/14 day intact male assays or short-term 28 day repeated dose studies

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CLA Concerns

- **At present, <u>insufficient</u> evidence to support extension/ additional animals in the 2-gen repro study solely for ED effects**
  - In utero exposure issue being evaluated in other forums
  - Coordinate with other EPA/ government programs
  - Consider international harmonization issues

- **Clarify use and value of one-generation rat in utero lactation assay**
  - Useful or confounding data?
  - Redundant with other tests?
  - How will these data be used to determine ED and or in risk assessment?
  - Is <u>not</u> a half of a 2-gen, assay, still needs to be demonstrated and validated

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